FRET-paired dyes and functionalized DNA handles using disulfide and click chemistry. These handles tether each molecule to the surface of a coverslip on one end and to a paramagnetic bead on the other. By simultaneously measuring donor and acceptor intensities, as well as magnet position, we are able to monitor force-induced conformational changes in multiple protein systems.

2374-Pos Board B66
Determining Solute Effects on Protein Folding using Single Molecule Force Spectroscopy
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In vivo, proteins function in a complex environment where they are subject to stresses like solutes, temperature and strain. Therefore, characterizing the effect of the external environment is essential to solving the protein folding problem. We use optical tweezers to explore the effect of two environmental factors, force and solutes, on protein folding. Optical tweezers are used to probe the response of single protein molecules to force along a specific pulling axis. Here, we pull on the src SH3 domain in the presence of the denaturant urea and the stabilizing osmolyte glycine betaine (GB) to create phase diagrams describing that state of src SH3 at different forces and urea or GB concentrations. Because solute effects are related to the amount and type of surface buried in a process, they can be used to probe conformational changes. Urea and GB are used to explore how conformational changes in single molecule force experiments and standard ensemble experiments differ. Effects of urea and GB on folding and unfolding rates when pulled along different axes provides information about conformational changes in folding to and from the transition state along different pathways. Moreover, solutes could perturb folding enough to reveal unexplored areas of the folding landscape, increasing our understanding of different pathways for folding.

2375-Pos Board B67
Exploring the Complex Energy Landscape of Protein Unfolding Under Force
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Optical tweezers have enabled the use of extremely low mechanical forces to study the unfolding and refolding of single protein biomolecules. We investigated the complex mechanical behavior of the src SH3 domain, a classical two-state folding protein. When a shearing force is applied on the terminal β-strand, the protein accesses parallel unfolding pathways, as indicated by the biphasic force dependence of the unfolding rates. We characterized the mechanical transition states for the two pathways by developing a protein engineering approach analogous to the phi-value methodology used in bulk studies. Interestingly, the transition state of the high-force unfolding pathway has more similarities with the zero-force, bulk unfolding pathway. The transition state is highly polarized under zero-force conditions and at high forces. However, at intermediate forces, the transition state is very diffuse, as indicated by a phi-value of ~0.5 for all the src SH3 variants. To the best of our knowledge, this study represents the first instance of an experimentally observed, force-dependent change in the malleability of the transition state structure.

2376-Pos Board B68
Is Refolding of Lysozyme Template-Driven
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Protein refolding is known to be dependent on factors such as protein concentration, temperature, pH, denaturant concentration, and several other factors. Protein folding and aggregation are competing reactions and the factors responsible for the partition between these two reactions are still not well understood. We have shown that lysozyme even at very high concentrations (10 mg/mL) is capable of refolding to near 100% activity when its disulfide bonds are intact. However, in the presence of a reducing agent such as DTT, lysozyme will aggregate significantly at very low concentrations (50 ug/mL). Furthermore, when refolding, denatured/reduced lysozyme is unable to refold in the absence of a redox mixture of reduced and oxidized glutathione. Previous studies conducted by Trivedi et. al have also demonstrated that the renaturation yield of lysozyme increases when co-refolding in the presence of other denatured states of basic proteins. We hypothesize that the renaturation yield of lysozyme will also increase when refolding in the presence of the native form of lysozyme. Furthermore, we believe that protein refolding is to a certain degree template-driven.

2377-Pos Board B69
High Throughput Screening of Formulations to Optimize the Thermal Stability of Therapeutic Monoclonal Antibody
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Monoclonal antibodies (mAb) are progressively more important class of biotherapeutics. Antibodies are heterodimers composed of two heavy and two light polypeptide chains linked by disulfide bonds. The majority of recombinant antibodies currently under development are IgGs. Successful development of therapeutic antibodies depends not only on attainment of desired biological activities but also on physicochemical properties of these molecules, such as homogeneity, monomer oligomeric state and stability enabling manufacturing, storage and delivery. Antibody stability is affected by its formulation: ionic strength, pH, buffering substance, presence of excipients and protein concentration. Among many techniques used to study the effects of formulation on stability of antibodies, differential scanning fluorimetry (DSF) is distinguished by an unprecedented throughput and minimal material consumption. DSF measures the midpoint of unfolding transition of protein - T_m - during thermal denaturation based on the change in fluorescence intensity of environmentally sensitive dye Sypro Orange.
Here, we analyzed the impact of formulation on stability of mAb1 using DSF adapted to the 96-well plate format. Two to three thermal unfolding transitions were visible for mAb1, which reproduced closely the melting profile obtained with differential scanning calorimetry (DSC). Good correlation was also observed between T_m of the main thermal unfolding transitions determined by DSF and DSC, with the former being on average lower by 3°C. Basal fluorescence of Sypro Orange was enhanced by the presence of detergents, limiting the use of this approach to the diluted detergent solutions. We have shown that low pH or high salt concentrations decreases the thermal stability of mAb1, whereas some excipients such as sucrose, methionine and phosphate buffer increase its stability. We have also shown that the throughput of DSF can be increased further with the use of 384-well plate.

2378-Pos Board B70
Conformational Analysis of ACTH/Melanocortin Precursor Protein
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Proopiomelanocortin (POMC), a precursor protein of ACTH, serves as the source of a large number of biologically active peptide hormones, including MSHs, CLIP, LPH, and endorphin. POMC is sequentially processed at pairs of basic amino acid residues via a sequence of enzymatic steps in a tissue-specific manner by pro-hormone convertase 1 and 2 (PC1/2) [1]. However, little is known regarding its own role or the machinery associated with its processing in detail, based on its tertiary structure.
To obtain structural information related to the mechanism associated with the processing of POMC, recombinant POMC was over-expressed in *E. coli* cells using the artificial gene encoding POMC which was optimized at the codons and GC contents [2]. Recombinant POMC was readily over-expressed as a soluble protein and purified using several types of chromatography, such as Ni-chelate affinity and hydroxyapatite chromatography. Circular dichroism (CD) measurements of the purified POMC were carried out under several different conditions, including pH and different solvents. The results suggested that recombinant POMC possesses less α-helical and β-sheet structure in aqueous solutions used in our experiments. However, the contents of the α-helical structure of POMC were increased in a concentration dependent manner when trifluoroethanol was used as the solvent. The thermodynamic stability of POMC was also evaluated under several conditions by CD measurements. The collective results will be discussed.
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2379-Pos Board B71
Contribution of Methionine Oxidation to Amyloid Fibril Formation by Apolipoprotein A-I
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Apolipoprotein A-I (apoA-I) is an important promoter of cardiovascular health. However, oxidation in physiological conditions can alter the structure/function of apoA-I and transform this atheroprotective protein into a potentially harmful